Neural Regulation of Endothelial Cell-Mediated Inflammation

Kimberly Quinlan Lindsey,*[1] S. Wright Caughman,* John E. Olerud,‡ Nigel W. Bunnett,†
Cheryl A. Armstrong,*§ and John C. Ansel*§

*Department of Dermatology and Emory Skin Disease Research Core Center, Emory University School of Medicine, Atlanta, Georgia, U.S.A.; †Departments of Surgery and Physiology, University of California San Francisco, San Francisco, California, U.S.A.; ‡Department of Dermatology, University of Washington, Seattle, Washington, U.S.A.; §Dermatology Service, Veterans Affairs Medical Center, Atlanta, Georgia, U.S.A.

There is increasing evidence that the cutaneous neurosensory system can directly modulate inflammatory responses in the skin by the release of neuropeptides such as substance P (SP). Dermal microvascular endothelial cell (DMEC) cellular adhesion molecule (CAM) expression plays a key role in directing leukocyte trafficking during cutaneous inflammatory responses. In recent studies, our laboratory examined the direct effect of SP on DMEC CAM expression and function *in vitro* and *in vivo*. Our studies indicate that DMEC express high affinity functional receptors for SP. After exposure to SP, DMEC expressed significant levels of both intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which was accompanied by increased binding to leukocytes expressing the appropriate integrin counter receptors for these CAM. We then determined the *in vivo* effect of released neuropeptides on DMEC CAM expression. Our results indicate that the topical cutaneous application of the neuropeptide-releasing agent capsaicin resulted in increased ICAM-1 and VCAM-1 immunostaining of microvascular cells in the skin of human volunteers. Little is known regarding the cellular regulatory events by which SP modulates DMEC CAM expression. Our studies indicate that SP-induced cellular Ca^{+2} signals led to the activation of the NF-κB pathway, resulting in nuclear translocation of p65/p50 heterodimers that bind to high-affinity tandem κB sites on the VCAM-1 promoter, whereas SP activation induced NF-AT activation and ICAM-1 DNA binding. Thus, these studies further support the role of the cutaneous neurologic system in modulating inflammatory processes in the skin.
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There is significant evidence that the cutaneous neurologic system can mediate inflammatory responses in the skin through the release of specific neuropeptides that can interact with various epidermal and dermal target cells. The close physical association of cutaneous nerves with these target cells has been clearly established (Weisner-Menzel *et al*, 1981; Wang *et al*, 1990; Hosoi *et al*, 1993). It has been appreciated that neuropeptides such as substance P (SP) and calcitonin gene related peptide (CGRP) have a number of vasoactive properties, including vasodilation, increased microvascular permeability, and protein extravasation (Erjavec *et al*, 1981; Brain *et al*, 1986). Additionally, SP can induce leukocyte effector activities such as lymphocyte proliferation, immunoglobulin production, cytotoxicity, mast cell degranulation, PMN and macrophage activation, and cytokine production by monocytes (Hartung and Toyka, 1983; Payan *et al*, 1983; Kimball *et al*, 1988; Lotz *et al*, 1988; Wiederman *et al*, 1989; McGillis *et al*, 1991; Ansel *et al*, 1996, 1997; Scholzen *et al*, 1998). We have previously shown that SP can directly activate mast cells and keratinocytes to secrete TNFα and IL-1, respectively (Brown *et al*, 1990; Ansel *et al*, 1993; Song *et al* 2000). We have recently examined the role of the cutaneous neurologic system in the modulation of certain proinflammatory functions by cutaneous microvascular endothelial cells.

A critical component of the initiation and evolution of localized inflammation is the homing and extravasation of leukocytes at the sites of tissue injury, which is fundamentally directed by the expression of cell adhesion molecules (Caughman, 1991; Carlos and Harlan, 1994). Leukocyte–endothelial adhesion interactions leading to extravasation are based on a sequential series of events requiring regulated expression of multiple adhesion proteins by endothelial cells including E-selectin, intercellular adhesion molecule 1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) (Rothlein *et al*, 1986; Bevilacqua *et al*, 1987; Rice and Munro, 1990). The assessment of cutaneous neuropeptide modulation of human dermal microvascular endothelial cell (HDMEC) cellular adhesion molecule (CAM) expression is important to our understanding of interactions between the cutaneous neurologic system and skin inflammatory responses. Indeed, HDMEC perform a key gatekeeper function in the initiation, modulation, and termination of inflammatory responses in the skin. Inflammatory infiltration of leukocytes into all tissues, including the skin, depends on leukocyte

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Reprint requests to: Dr. John C. Ansel, Department of Dermatology, 5001 Woodruff Memorial Building, Emory University School of Medicine, Atlanta, GA 30322. Email: jansel@emory.edu

[1]Present address: Centers for Disease Control and Prevention, Atlanta, GA 30333, U.S.A.

passage into tissue from the microvasculature in a multi-step binding interaction involving CAM expressed by leukocytes and endothelial cells (Lawrence and Springer, 1991; von Andrian *et al*, 1991). P-selectins and E-selectins mediate initial leukocyte adhesion of HDMEC through binding to specific carbohydrate complexes present on neutrophils, whereas ICAM-1 and VCAM-1 mediate subsequent firm adhesion and transendothelial migration by binding to β2 and β1-integrins on leukocytes.

SP REGULATION OF HDMEC ICAM-1 EXPRESSION AND FUNCTION

In the skin, HDMEC expression of ICAM-1 is an essential component of cutaneous inflammation. ICAM-1 is constitutively expressed *in vitro* by HDMEC. The proinflammatory cytokines, interferon-γ, TNFα, and IL-1 as well as UVB irradiation, increase ICAM-1 surface expression (Swerlick *et al*, 1991; Cornelius *et al*, 1994). Understanding how neuropeptides regulate HDMEC surface ICAM-1 expression will advance our knowledge of important initiating events in cutaneous inflammation. In a previous study, it was reported that SP was capable of upregulating ICAM-1 on cultured large vessel human umbilical vein endothelial cells (HUVEC). This study did not, however, examine the effect of SP on dermal microvascular endothelial cells *in vitro* or *in vivo*, and neuropeptide receptors were not characterized (Nakagawa *et al*, 1993). Because all tissue inflammatory responses in the skin and elsewhere are mediated by microvascular endothelial cell activities rather than by large vessel endothelial cells, and because the two cell types display distinct differences in their phenotypes and responses to proinflammatory signals (Swerlick and Lawley, 1993), it is essential to measure neuroinflammatory responses in HDMEC (Quinlan *et al*, 1998).

Recent studies in our laboratory indicate that cultured HDMEC express mRNA for the neurokinin receptors NK1R, NK2R, and NK3R, which are capable of binding to SP with high, intermediate, and low affinities, respectively (Quinlan *et al*, 1998). Our studies indicate that SP induces a rapid intracellular Ca^{+2} response in HDMEC and that this effect is mediated primarily by NK1R. *In vivo*, immunohistochemistry studies demonstrate that the NK1R is the major neurokinin receptor expressed on dermal microvascular endothelial cells. HDMEC activation by SP is accompanied by increased levels of ICAM-1 mRNA and ICAM-1 cell surface expression. The optimal increase in HDMEC ICAM-1 occurs 16–18 h after exposure to SP. Flow cytometric analysis of ICAM-1 expression of SP-treated HDMEC indicates that induction is mediated primarily by the NK1R (**Fig 1**). The functional consequences of SP induction of cell surface ICAM-1 on binding of J-Y lymphoblastoid cells to HDMEC were determined *in vitro* using a quantitative cellular adhesion assay. Our results indicate that the addition of SP resulted in a significant increase in J-Y cell adhesion to cultured HDMEC. The adhesion of these leukocytes to SP-treated HDMEC could be prevented by pretreatment of HDMEC with an anti-ICAM-1 blocking antibody before the addition of labeled J-Y cells.

The effect of *in vivo* release of cutaneous neuropeptides on HDMEC ICAM-1 expression was also examined. A capsaicin containing cream, Zostrix, was applied to the skin of human volunteers to stimulate the release of cutaneous neuropeptides including SP from cutaneous sensory nerves (**Fig 2**). Low constitutive levels of microvascular ICAM-1 expression were observed in untreated skin. Microvascular ICAM-1 expression gradually increases at 6 h, markedly increases by 24 h, and remains upregulated even at 48 h after topical application of capsaicin. Thus, the *in vivo* release of neuropeptides by cutaneous sensory nerves results in increased microvascular endothelial ICAM-1 expression.

Thus, our studies demonstrated that SP is capable of directly modulating HDMEC ICAM-1 expression and function by the activation of specific cell surface neurokinin receptors. There is also evidence that SP can mediate indirect induction of endothelial cell

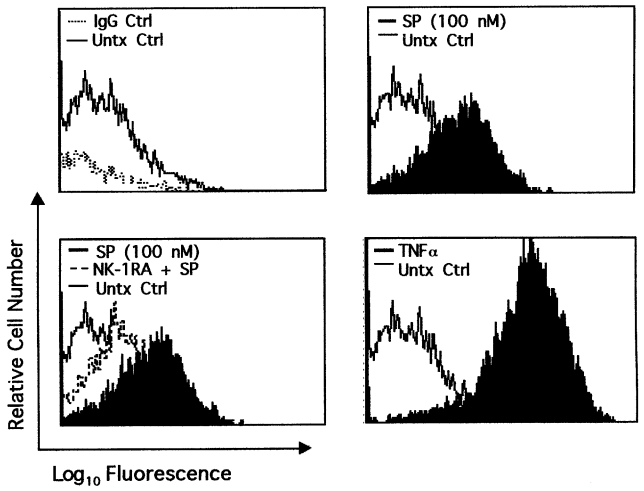

Figure 1. Flow cytometric analysis of ICAM-1 expression of SP-treated HDMEC. Surface expression of ICAM-1 on HDMEC was also assessed by flow cytometric analysis at 18 h after exposure to NK-1RA followed by 100 nM SP (bottom left), 100 nM SP (top right), or 300 μ per ml TNFα (bottom right). Open histogram areas under solid lines represent constitutive ICAM-1 expression (Untx Ctrl); superimposed filled histogram areas represent expression with treatments indicated by heavier solid lines in keys. Top left shows untreated cells incubated with an isotype control IgG in place of the anti-ICAM-1 antibody. The data are representative of experiments conducted in triplicate. (Published in Quinlan *et al*, 1998.)

CAM expression. Murphy and others have demonstrated that SP treatment of skin explants caused mast cell degranulation and subsequent TNFα mediated induction of E-selectin on postcapillary venular endothelial cells (Matis *et al*, 1990). In active cutaneous inflammation, we therefore propose that SP may influence dermal microvascular cell CAM expression both directly and indirectly through the activation of other target cells in the skin.

SP DIRECTLY AND SPECIFICALLY UPREGULATES VCAM-1 EXPRESSION ON HDMEC

In the skin, the expression of specific combinations of adhesion molecules is closely regulated and correlates with the selective recruitment of leukocyte subtypes. The biologic functions of adhesion molecules ICAM-1 and VCAM-1 are distinct. Unlike ICAM-1, which is widely distributed on antigen presenting cells, epithelial cells, and fibroblasts, in the skin cell surface expression of VCAM-1 is mainly restricted to the vascular endothelium (Elangbam *et al*, 1997), though its expression and role on other cell types in other tissues has been described (Iademarco *et al*, 1993; Lukacs *et al*, 1994; Yellin *et al*, 1995). Therefore, we also examined the effect of different cutaneous nerve-released neuropeptides on microvascular endothelial cell VCAM-1 expression and function (Quinlan *et al*, 1999a).

To determine the *in vivo* effect of C-fiber released neuropeptides on HDMEC VCAM-1 expression, capsaicin cream was applied topically to human volunteers to stimulate the release of neuropeptides including SP. Our results indicate that increased VCAM-1 immunoreactivity could be detected by 6 h after capsaicin application, with peak expression at 24 h and decreased expression 48 h after treatment. Thus, the release of neuropeptides by cutaneous sensory nerves results in increased HDMEC VCAM-1 expression *in vivo*.

Because a number of neuropeptides are detected in normal and inflamed skin, the direct effect of these different neuropeptides on HDMEC VCAM-1 was tested *in vitro* (**Fig 3**) (Quinlan *et al*, 1999a). Untreated HDMEC express little or no cell surface VCAM-1. SP was able to induce a dose dependent increase (up to 7-fold) in HDMEC VCAM-1 expression. Optimal expression of

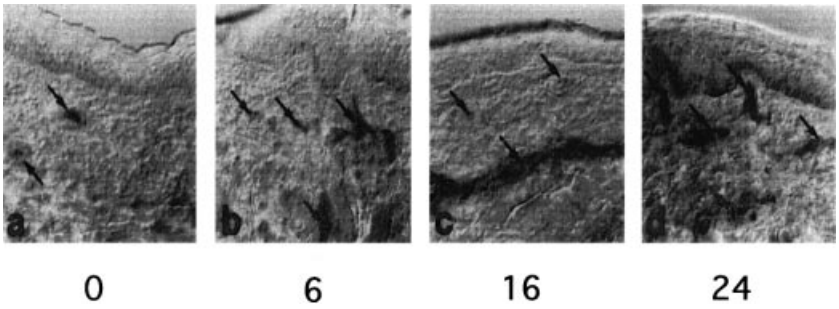

Figure 2. *In vivo* induction of HDMEC ICAM-1 expression. Photomicrographs were taken of human skin biopsies immunostained for ICAM-1 expression after topical capsaicin application to release cutaneous neuropeptides. *Arrows* point to dermal microvascular structures. Human skin was left untreated (*A*) or treated with capsaicin cream (0.075%) and biopsied at 6 h (*B*), 24 h (*C*), or 48 h (*D*). (Published in Quinlan *et al*, 1998.)

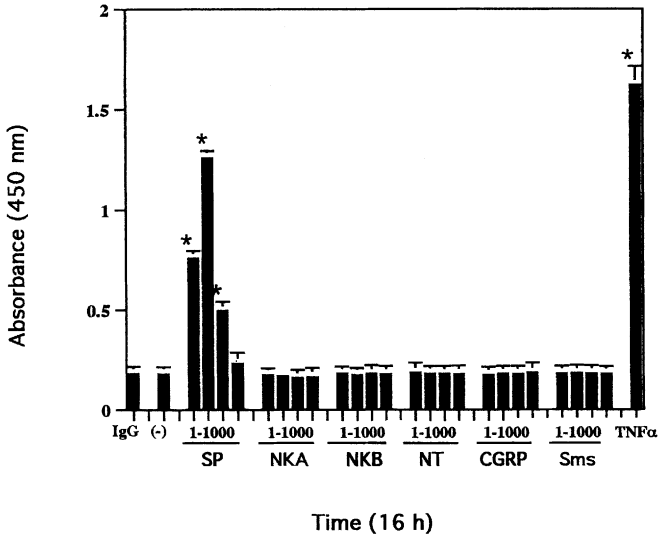

Figure 3. ELISA measurement of VCAM-1 expression of neuropeptide-treated HDMEC. HDMEC were stimulated with 1, 10, 100, or 1000 nM of either SP, neurokinin A (NKA), neurokinin B (NKB), neurotensin (NT), calcitonin gene-related peptide (CGRP), or somatostatin (Sms). TNFα, 300 U per ml, was utilized as a positive control. Incubations were for 16 h at 37°C and cell surface VCAM-1 was measured by ELISA. Statistically significant differences in cell surface VCAM-1 in treated samples as compared with untreated control cells (–) were determined by Student's t test as indicated by (*) ($p < 0.005$). The data shown are representative of experiments conducted in triplicate. (Published in Quinlan *et al*, 1999a.)

HDMEC VCAM-1 occurred 16 h after the addition of SP. In contrast, under similar experimental conditions, incubation with neurokinin A (NKA), neurokinin B (NKB), neurotensin (NT), calcitonin gene related peptide (CGRP), and somatostatin (SMA) had no effect on HDMEC VCAM-1 induction. We then determined if SP directly upregulates the expression of HDMEC VCAM-1 or whether this was in part due to the induction by SP of HDMEC-secreted soluble factors that in turn could be responsible for increased VCAM-1 expression. Our studies indicate that the induction of HDMEC VCAM-1 expression could be completely abrogated by the addition of anti-SP antibodies to the culture supernatant of SP-treated HDMEC, which strongly supports the direct effect of SP on HDMEC VCAM-1 expression.

The biologic consequence of SP-induced HDMEC VCAM-1 cell surface expression was determined using a quantitative cellular adhesion assay with Molt-4 T cells that express the specific counter receptor ligand for VCAM-1. The addition of 10 nM SP induced a 4-fold increase in the adhesion of Molt-4 cells to HDMEC, which was blocked by pretreating cells with a specific antibody to VCAM-1. Thus, these studies further support the role of neuropeptides in the regulation of leukocyte trafficking in the skin.

TRANSCRIPTIONAL MECHANISMS BY WHICH SP MODULATES CELLULAR ADHESION MOLECULE GENE EXPRESSION IN HDMEC

SP mediates its effects on target cells by binding to cell surface G-protein coupled neurokinin receptors. SP binds to a high-affinity receptor, NK1R, which we have detected in HDMEC. Engagement of the NK1R with SP results in an increase in intracellular Ca^{+2} levels, followed by an increase in expression of HDMEC ICAM-1 and VCAM-1. As little is known of the regulatory events by which SP modulates adhesion gene expression, however, we have conducted gene transcription studies to better understand this mechanism (Quinlan *et al*, 1999b).

The final targets of cell surface signals are often the activated proteins that associate with DNA regulatory elements and regulate transcription. ICAM-1 and VCAM-1 are highly regulated at the transcriptional level by a number of mediators (Neish *et al*, 1992; Marui *et al*, 1993; Cornelius *et al*, 1994; Gille *et al*, 1996; Duff *et al*, 1997; Paxton *et al*, 1997). Transcription of ICAM-1 and VCAM-1 induced by TNFα is modulated by distinct members of the Rel family. NF-κB is retained in the cytoplasm in an inactive form by the inhibitory protein IκB, which in turn is regulated by the IκB kinase, IKK (Israel, 1997). Following a variety of extracellular stimuli, NK-κB dissociates from IκB, translocates to the nucleus, and activates a number of target genes. The minimal DNA binding domain of NF-κB (p65/p50) on the ICAM-1 promotor corresponds exactly to the NF-AT high-affinity consensus site (TGGAAA) (Prieschl *et al*, 1995; Rooney *et al*, 1995; Tsytsykova *et al*, 1996). Within particular NF-κB DNA binding domains, an NF-AT monomer can bind to the 5′ half-site of the DNA binding domain, and a second NF-AT monomer can bind to the symmetrical 3′ half-site, which is why NF-AT transcription factors are often termed monomeric Rel proteins (Rao *et al*, 1997). NF-AT is expressed in most immune cells and like NK-κB, plays a pivotal role in the transcription of genes critical for inflammatory responses.

We have identified the SP-activated transcription factors that associate with the DNA binding domains of the ICAM-1 and VCAM-1 regulatory regions in HDMEC (**Fig 4**). Our studies have demonstrated SP-mediated activation results in both NF-AT binding to the –191/–170 of ICAM-1 and NF-κB (p65/p50) binding to the –76/–52 of VCAM-1 genes. These events were specifically blocked by both the NK1R antagonist and by cyclosporine A (CsA). Although NF-AT is associated with heterologous DNA binding proteins, especially AP-1, SP-driven NF-AT/ICAM-1 appears *in vitro* to lack cooperative binding with

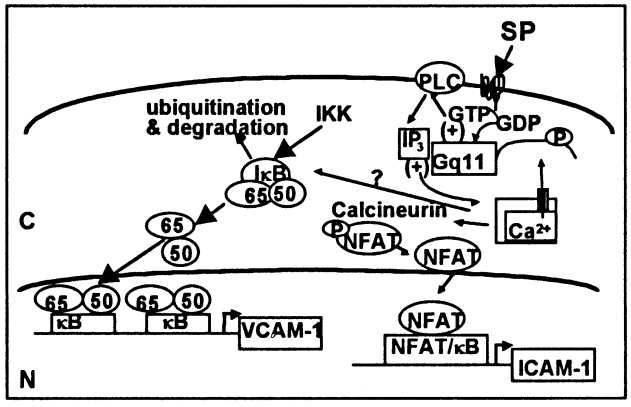

Figure 4. Model for SP-induced differential regulation of ICAM-1 and VCAM-1 gene expression in HDMEC. ICAM-1 transcription in response to SP is activated via NFAT activation and binding to a modified NFκB site that is critical to activation of ICAM-1 expression in response to TNFα. TNFα activation is mediated via activation and binding to this site by p65 homodimers and not NFAT. For VCAM-1 transcription activation via SP, the tandem NFκB sites critical to both TNFα and IL-1 VCAM-1 upregulation, are also utilized for SP activation of transcription, and the transcription factors involved in this case are indeed the common NFκB heterodimer, p65/p50. For activation of both ICAM-1 and VCAM-1 transcription via SP, transcription is directly linked to calcium ion mobilization, which is a completely new pathway of activation for NFκB, though this pathway has long been known to be utilized for NFAT activation. (Published in Quinlan *et al*, 1999b.)

transcription factors of the AP-1 family. While it is possible that cooperation with other transcription factors may occur *in vivo*, our site-directed mutagenesis studies show that the NF-AT site, which overlaps with the 5′ portion of the p65 homodimer NF-κB binding site, abrogates both SP- and TNFα-induced transcription, even within the context of the remaining native ICAM-1 promoter. Our data also indicate that SP-elicited intracellular Ca^{+2} mobilization is required for both NF-AT and NF-κB activation and binding to their respective consensus sites on the ICAM-1 and VCAM-1 genes. This is in contrast to the TNFα-activated NF-κB pathway, which is Ca^{+2} independent. Thus, this provides further evidence that SP and TNFα modulate CAM transcription via distinct signaling pathways. There are recent reports of the requirement of intracellular Ca^{+2} mobilization for NF-κB activation and κB-dependent gene expression in other cell types and by signals other than neuropeptides (Pahl *et al*, 1996; Sun and Carpenter, 1998).

CONCLUSION

HDMEC are essential participants of the human cutaneous inflammatory system. These cells express both soluble and cell-associated proinflammatory molecules that play a key role in the recruitment and trafficking of various types of leukocytes into the dermis and epidermis during inflammatory skin diseases. The expression and function of HDMEC ICAM-1 and VCAM-1 seem to be required for normal leukocyte responses in the skin by facilitating adhesion and transmigration into the extravascular compartment of the skin. Our recent studies demonstrate that released neuropeptides such as SP can directly modulate the expression of HDMEC CAM both *in vitro* and *in vivo*. Thus, our studies further support the concept that the cutaneous neurologic system can regulate key aspects of inflammatory processes in the skin.

REFERENCES

Ansel J, Brown J, Payan D, Brown M: Substance P selectively activates TNF-α gene expression in murine mast cells. *J Immunol* 150:1–8, 1993

Ansel JC, Kaynard AH, Armstrong CA, Olerud J, Bunnett N, Payan D: Skin–nervous system interactions. *J Invest Dermatol* 106:198–204, 1996

Ansel JC, Armstrong CA, Song IS, Quinlan KL, Olerud JE, Caughman SW, Bunnett NW: Interactions of the skin and nervous system. *J Invest Dermatol Symp Proc The* 2:23–26, 1997

Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA Jr: Identification of an inducible endothelial-leukocyte adhesion molecule. *Proc Natl Acad Sci* 84:9238–9242, 1987

Brain SD, Tippins JR, Morris HR, MacIntyre I, Williams TJ: Potent vasodilator activity of calcitonin gene-related peptide in human skin. *J Invest Dermatol* 87:533–536, 1986

Brown J, Perry P, Hefeneider S, Ansel J. Neuropeptide modulation of keratinocyte cytokine production. In: Oppenheim JJ, Powanda MC, Kluger MJ, Dinarello CA, eds. *Molecular and Cellular Biology of Cytokines*. New York: Wiley-Liss, 1990, pp. 451–456

Carlos TM, Harlan JM: Leukocyte-endothelial adhesion molecules. *Blood* 84:2068–2101, 1994

Caughman SW: Adhesion molecules: their roles in cutaneous biology and inflammation. *Prog Dermatol* 25:1–8, 1991

Cornelius LA, Sepp N, Li L-J, Degitz K, Swerlick RA, Lawley TJ, Caughman SW: Selective upregulation of intercellular adhesion molecule (ICAM-1) by ultraviolet B in human dermal microvascular endothelial cells. *J Invest Dermatol* 103:23–28, 1994

Duff JL, Quinlan KL, Paxton LL, Naik SM, Caughman SW: Pervanadate mimics interferon γ-mediated induction of ICAM-1 expression via activation of STAT proteins. *J Invest Dermatol* 108:295–301, 1997

Elangbam CS, Qualls CW Jr, Dahlgren RR: Cell adhesion molecules: an update. *Vet Pathol* 34:61–73, 1997

Erjavec F, Lembeck F, Florjanc-Irman T: Release of histamine by substance P. Naunyn Schmiedebergs. *Arch Pharmacol* 317:67–70, 1981

Gille J, Swerlick RA, Lawley TJ, Caughman SW: Differential regulation of vascular cell adhesion molecuel-1 gene transcription by tumor necrosis factor alpha and interleukin-1 alpha in dermal microvascular endothelial cells. *Blood* 87:211–217, 1996

Hartung H, Toyka K: Activation of macrophages by substance P. induction of oxidative burst and thromboxane release. *Eur J Pharmacol* 89:301–305, 1983

Hosoi J, Murphy G, Egan C, Lerner E, Grabbe S, Asahina A, Granstein R: Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. *Nature* 363:159–163, 1993

Iademarco MF, McQuillan JJ, Dean DC: Vascular cell adhesion molecule 1: contrasting transcriptional control mechanisms in muscle and endothelium. *Proc Natl Acad Sci* 90:3943–3947, 1993

Israel A: IκB kinase all zipped up. *Nature* 388:519–521, 1997

Kimball ES, Persico FJ, Vaught JL: Substance P, neurokinin A, and neurokinin B induce generation of IL-1 like activity in P388D1 cells. *J Immunol* 141:3564–3569, 1988

Lawrence MB, Springer TA: Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. *Cell* 65:859–873, 1991

Lotz M, Vaughan JH, Carson DA: Effect of neuropeptides on production of inflammatory cytokines by human monocytes. *Science* 241:1218–1221, 1988

Lukacs NW, Strieter RM, Evanoff HL, Burdick MD, Kunkel SL: VCAM-1 influences lymphocyte proliferation and cytokine production during mixed lymphocyte response. *Cell Immunol* 154:88–98, 1994

Marui N, Offermann MK, Swerlick R, *et al*: Vascular cell adhesion molecule-1 (VCAM-1) gene transcription and expression are regulated through an antioxidant-sensitive mechanism in human vascular endothelial cells. *J Clin Invest* 92:1866–1874, 1993

Matis WL, Lavker RM, Murphy GF: Substance P induces the expression of an endothelial-leukocyte adhesion molecule by microvascular endothelium. *J Invest Dermatol* 94:492–495, 1990

McGillis J, Mitsuhashi M, Payan D. Immunologic properties of substance P. In: R Ader, D Felten, N Cohen, eds. *Psychoneuroimmunology*. San Diego: Academic Press, 1991, pp. 209–223

Nakagawa N, Iwamoto I, Yoshida S: Effect of substance P on the expression of an adhesion molecule ICAM-1 in human vascular endothelial cells. *Reg Peptides* 46:223–224, 1993

Neish AS, Williams AJ, Palmer HJ, Whitley MZ, Collins T: Functional analysis of the human vascular cell adhesion moleucle-1 promoter. *J Exp Med* 176:1583–1593, 1992

Pahl HL, Sester M, Burgert H-G, Baeuerle PA: Activation of transcription factor NF-κB by the adenovirus E3/19K protein requires its ER retention. *J Cell Biol* 132:511–522, 1996

Paxton LLL, Li L-J, Secor V, Duff JL, Naik SM, Shibigaki N, Caughman SW: Flanking sequences for the human intercellular adhesion molecule-1 NF-kappa B response element are necessary for tumor necrosis factor alpha-induced gene expression. *J Biol Chem* 272:15928–15935, 1997

Payan D, Brewster D, Goetzl E: Specific stimulation of human T lymphocytes by substance P. *J Immunol* 131:1613–1615, 1983

Prieschl EE, Gouilleux-Gruart V, Walker C, Harrer NE, Baumruker T: A nuclear factor of activated T cell-like transcription factor in mast cells is involved in IL-5 gene regulation after IgE plus antigen stimulation. *J Immunol* 154:6112–6119, 1995

Quinlan KL, Song I-S, Bunnett NW, *et al*: Neuropeptide regulation of human dermal microvascular endothelial cell ICAM-1 expression and function. *Am J Physiol* 275:C1580–C1590, 1998

Quinlan KL, Song I-S, Naik SM, *et al*: VCAM-1 expression on human dermal microvascular endothelial cells is directly and specifically up-regulated by substance P. *J Immunol* 162:1656–1661, 1999a

Quinlan KL, Naik SM, Cannon G, Armstrong CA, Bunnett NW, Ansel JC,

Caughman SW: Substance P activates coincident NF-AT- and NF-κB-dependent adhesion molecule gene expression in microvascular endothelial cells through intracellular calcium mobilization. *J Immunol* 163:5656–5665, 1999b

Rao A, Luo C, Hogan PG: Transcription factors of the NFAT family: regulation and function. *Annu Rev Immunol* 15:707–747, 1997

Rice GE, Munro JM: Bevilacqua MP: Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes. A CD11/CD18-independent mechanism. *J Exp Med* 171:1369–1374, 1990

Rooney JW, Sun YL, Glimcher LH, Hoey T: Novel NFAT sites that mediate activation of the interleukin-2 promoter in response to T-cell receptor stimulation. *Mol Cell Biol* 15:6299–6310, 1995

Rothlein R, Dustin ML, Marlin SD, Springer TA: A human intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J Immunol* 137:1270–1274, 1986

Scholzen T, Armstrong CA, Bunnett NW, Luger TA, Olerud JE, Ansel JC: Neuropeptides in the skin: interactions between the neuroendocrine and the skin immune systems. *Exp Dermatol* 7:81–96, 1998

Song I, Bunnett NW, Olerud JE, *et al*: Substance P induction of murine keratinocyte PAM 212 interleukin 1 production is mediated by the neurokinin 2 receptor (NK-2R). *Exp Dermatol* 9:42–52, 2000

Sun L, Carpenter G: Epidermal growth factor activation of NF-κB is mediated through IκBα degradation and intracellular free calcium. *Oncogene* 16:2095–2102, 1998

Swerlick RA, Garcia-Gonzolez E, Kubota Y, Xu Y, Lawley TJ: Studies of the modulation of MHC antigen and cell adhesion molecule expression on human dermal microvascular endothelial cells. *J Invest Dermatol* 97:190–196, 1991

Swerlick RA, Lawley TJ: Role of microvascular endothelial cells in inflammation. *J Invest Dermatol* 100:111S–115S, 1993

Tsytsykova AV, Tsitsikov EN, Geha RS: The CD40L promoter contains nuclear factor of activated T cells-binding motifs which require AP-1 binding for activation of transcription. *J Biol Chem* 271:3763–3770, 1996

von Andrian UH, Chambers JD, McEvoy LM, Bargatze RF, Arfors K-E, Butcher EC: Two-step model of leukocyte–endothelial cell interaction in inflammation: distinct roles for LECAM-1 and the leukocyte β2 integrins in vivo. *Proc Natl Acad Sci* 88:7538–7542, 1991

Wang L, Hilliges M, Jernberg T, Wiegleb-edstrom D, Johansson O: Protein gene product 9.5-immunoreactive nerve fibres and cells in human skin. *Cell Tissue Res* 261: 25–33, 1990

Weisner-Menzel L, Schultz B, Vakilzadeh F, Czarnetzki BM: Electron microscopic evidence for a direct contact between nerve fibres and mast cells. *Acta Dermatol Venerol* 61:465–469, 1981

Wiederman C, Wiederman F, Apperl A, Kieselbach G, Konwalinka G, Braunsteiner H: In vitro human polymorphonuclear leukocyte chemokinesis and human monocyte chemotaxis are different activities of aminoterminal and carboxyterminal substance P. Naunyn Schmiedebergs. *Arch Pharmacol* 340:185–190, 1989

Yellin MJ, Winidoff S, Fortune SM, Baum D, Crow MK, Leterman S, Chess L: Ligation of CD40 on fibroblasts induces CD54 (ICAM-1) and CD106 (VCAM-1) upregulation and IL-6 production and proliferation. *J Leuk Biol* 58:209–216, 1995